

REVIEW ARTICLE

APOPTOSIS IN HEALTH AND DISEASE

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Abstract: Two distinct forms of cell death are known, necrosis which results from physical or chemical insult and apoptosis or programmed cell death results from programming within the cell for self destruction in response to internal and external stimuli. Apoptosis is a genetically governed process of cell death occurring in development and maintenance of multicellular organisms. It occurs to get rid of individual cells that become unwanted for various reasons or that present a threat to the organism. It is accompanied by distinct morphological changes. DNA fragmentation in most cases, and appears to be caused by the activities of specific genes. Its defective regulation may play a part in the aetiology of cancer, AIDS, autoimmune and degenerative diseases. Apoptosis offers potential for prevention and therapeutic modulation of these disorders.

Key words: apoptosis cell death genes cancer

INTRODUCTION

Eukaryotic cells die by one of the two stereotypic processes, necrosis or apoptosis. In necrosis the cell is killed by the hostile microenvironment whereas in apoptosis the cell is activated by specific signals to commit suicide in a normal environment. Apoptosis a widespread biological phenomenon is a genetically governed active process of cell death. The cell death appears to be fixed developmentally and is thus programmed, and hence also called programmed cell death. A multicellular organism must keep a balance between proliferation and cell death to maintain the proper number of cells. Thus during development a large number of cells die by apoptosis. In fact the original notion of apoptosis came from embryonic development studies.

Cell death occurs during normal development and homeostasis because the cells become unwanted for various reasons: 1) cells that have been evolutionary vestiges and seem to have no function e.g. interdigit cell death has been altered repeatedly in evolution to help adapt the shape of the foot to new microenvironment, 2) cells generated in excess e.g. many vertebrate neurons; sometimes these excess cells provide the organism with developmental plasticity, 3) cells that develop improperly e.g. in visual system of

developing vertebrate, cell death preferentially eliminates neurons with improper connections, 4) cells that have already functioned e.g. resorption of tail of tadpole and 5) cells that are harmful and must be eliminated to protect the animal e.g. death of immature T cells in mouse thymus. An organism must also remove senescent, damaged or abnormal cells that could interfere with organ function or develop into tumors. There is potential significance of apoptosis because of its involvement in embryogenesis, differentiation, aging, metamorphosis, neural development, epithelial turnover like that of the skin and gut lining, tumor regression etc. Apoptosis is also observed on withdrawal of trophic hormone from its target tissues and also in atrophy of prostate after castration. Cytotoxic effects of glucocorticoids on immature thymocytes and of tumor necrosis factor (TNF- α) on susceptible malignant cells, are also due to apoptosis (1).

Abnormalities in the regulation of cell death may contribute to the pathogenesis of both degenerative and neoplastic disease. Horvitz (2) was first to show apoptosis *in vivo*, even if it is in the worm *Caenorhabditis elegans*. He showed that out of the 1090 cells in the worm, 131 cells die apoptotically as the worm sculpts itself to maturity and that activation of cell death genes *ced-3* and *ced-4* seemed necessary

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for apoptosis whereas ced -9 was suppressor of apoptosis.

Three main sets of events have been described in the dying apoptotic cell: 1) distinct morphological changes like cell shrinkage, compactation of the chromatin, early collapse of nucleus, nuclear and cytoplasmic budding to form membrane bound apoptotic bodies, which are rapidly phagocytosed by adjacent cells or macrophages (3). Ingestion of apoptotic cells by macrophages does not release toxic contents into extracellular space, and unlike necrosis is not accompanied by inflammatory response, thus does not damage the adjacent cells, 2) The molecular/biochemical events involve fragmentation of nuclear DNA into 180 base pair multiples corresponding to the size of a nucleosome. Most reports suggest the involvement of a Ca^{2+} Mg^{2+} endonuclease in this cleavage response (4), 3) There is no uniform sequence of metabolic events that occur during apoptosis, but many upstream and downstream molecules have been implicated such as receptor binding and phosphorylation. In most cell types, an early increase in cytosolic ionized calcium is noted (5) which in turn can activate the nuclear endonuclease that cleaves DNA, and/or a transglutaminase that crosslinks cytosolic proteins (6). Calcium dependent proteases like calpain may also degrade the cytoskeleton. Apoptosis can often be inhibited or delayed by RNA synthesis inhibitors like actinomycin D, or protein synthesis inhibitors like cycloheximide (7). It depends on the cell type in question and the apoptotic stimulus. Thus most cells contain regulatory protein that can either inhibit or promote apoptosis. A variety of apoptotic modulators have been described in different systems. They include zinc ion, calcium chelators, agents that stimulate protein kinase C e.g. phorbol ester PMA, or interfere with calcium/calmodulin pathway, protein and RNA synthesis blockers, drugs that inhibit poly ADPR polymerase i.e. 3 amino-benzamide, polyamines, and cyclosporin (8).

Apoptosis and cancer :

Many carcinogens damage DNA and the cell in turn responds to this insult by either repairing the damage prior to replicating, or progresses through cell growth cycle, or undergoes apoptosis. Thus cells death by apoptosis prevents malignant transformation whereas abnormal apoptosis can promote cancer development. We still do not know what factors determine as to which of the above pathways are to be followed by the cell.

Cell death by apoptosis is an active process and appears to be caused by the activities of specific genes that act within the dying cells. The product of these genes might be synthesized only after a cell has been determined to die or alternatively these products though present, become activated only in cells undergoing apoptosis. Interestingly some of the immediate early genes that stimulate proliferation such as c-myc, c-fos and c-jun also appear to be increased in apoptosis (9). Their overexpression induces apoptosis in fibroblasts. Many human cancers have mutation or deletion in the p53 gene. This gene is not only an inhibitor of cell division but also a mediator of apoptosis (10). Among the suppressor gene for apoptosis is the bcl-2 oncogene. Hemopoietic, lymphoid cells, many epithelial cells and neurons contain bcl-2. Follicular B-cell lymphocytes that overexpress bcl-2 have a longer survival in culture on growth factor deprivation and are also resistant to radiation and glucocorticoid induced apoptotic cell death. Epstein Barr virus proteins increase the expression of bcl-2 in Burkitts lymphoma cells (11). Many different categories of diverse chemotherapeutic agents such as topoisomerase inhibitors, antimetabolites, alkylating agents, and hormone antagonists kill sensitive cells by apoptosis (12). Thus antitumour approaches need to be re-examined in the light of apoptosis.

Apoptosis and the immune system :

Cell death is an important component in the normal functioning of the immune system (13, 14). The immune system also appears to sculpt its antigen defending system by first building a large T cell population and then selectively killing only autoreactive T cells by clonal selection, a situation similar to embryonic development. The cells seem to die silently without provoking any kind of immune response. This is because the T cell receptor can recognize self-antigens present within the mouse and if these cells are allowed to survive and proliferate, an autoimmune disorder could develop. *In vitro* the death of entire population of immature thymocytes can be induced with glucocorticoids, radiation etc. (15). B lymphocytes have also been shown to undergo apoptosis but most studies have been performed on T lymphocytes and T-cells hybridomas.

TNF- α , a 17 KDa protein triggers apoptosis by binding to its receptor. It is predominantly secreted by

activated macrophages (16). We studied the molecular mechanism of cell killing induced by TNF- α in the sensitive and resistant variants of mouse epidermal JB6 cells. Morphological and biochemical changes characteristic of apoptosis were found to precede TNF- α induced cell death in TNF- α sensitive but not TNF- α resistant cells. The findings suggested that TNF- α induced cell killing via apoptosis in TNF sensitive cells and that a preferential and transient increase in c-Jun dephosphorylation and AP-1 transcriptional activity might contribute to the preferential apoptotic response in these cells (17). CTL mediated DNA fragmentation is similar to that described for developmental or TNF- α induced cell death, except the kinetics of its appearance is very fast, as early as 5 min after adding CTL to their targets (18). The antiviral role of cytotoxic T cells might include the destruction of the virus in the host cell by fragmentation of viral DNA (19). Some effector T cells might destroy viruses within target cells, through limited nucleic acid fragmentation, even without target cell lysis (20) and no requirement for new macromolecular synthesis. Senescence of neutrophil polymorphs following deprivation of growth factors also involve apoptosis (21, 22).

Apoptosis appears to play a role in autoimmune diseases. Autoreactive T & B lymphocytes can be generated throughout the life span due to random gene recombination and mutation but normally, immature lymphocytes that bind autoantigens die by apoptosis (23). However, defective deletion of these lymphocytes by apoptosis could predispose to autoimmunity. Fas antigen is a surface protein having homology with TNF- α and nerve growth factor receptor and mediates apoptosis (24). It is expressed in various human cells, including myeloid cells, T lymphoblastoid cells, diploid fibroblasts, in thymus, ovary, liver and heart (25). Mice carrying the lymphoproliferative mutation (lpr) have defects in Fas antigen gene. The lpr seems to interfere with T cell maturation and leads to lymphadenopathy with systemic lupus type of autoimmune disease, indicating an important role for Fas antigen in the negative selection of autoreactive T cells. Mouse anti Fas monoclonal antibody has cytolytic activity on human cells that express the antigen (26). Mouse T cell lymphoma transformed with human Fas antigen cDNA were killed by the anti Fas antibody by apoptosis.

Apoptosis in AIDS :

Apoptosis has been suggested to have evolved as

antiviral defense (27). A kind of pathological imbalance between rate of CD4 cell death and cell replacement has been seen in AIDS (28). Depletion of CD4 T lymphocytes leads to immunodeficiency in HIV-1 infected persons. It has been suggested that both qualitative and quantitative defects in CD4 T cells in patients with HIV infection may be the result of activation induced cell death or apoptosis (29). There is speculation that cross linking of CD4, molecules to one another by gp 120 alone or gp 120 in complex with anti gp 120 antibodies prepares the cell for programmed cell death that occurs when an MHC class II molecule in complex with antigen binds to the T cell antigen receptor. Thus, the mere activation of a preparatory cell by a specific antigen or superantigen could lead to the death of the cell, without direct infection by HIV. The apoptosis hypothesis would help explain the depletion of CD4 T cells without requiring that each depleted cell be infected with HIV-1. AIDS patients may have high levels of antibody to APO- 1/ Fas and it is a factor in T cell death. Thus apoptosis offers potential in AIDS therapy, the onset of immunodeficiency can be delayed by blocking an important/essential step of cell death by apoptosis.

Apoptosis in degenerative disease :

The removal of trophic factors and excessive exposure to excitatory amino acid neurotransmitters are toxic to neurons. On deprivation of nerve growth factor, the sympathetic neurons undergo apoptosis, but the high levels of bcl -2 in these neurons prevents apoptosis (30). Dying neurons have an apoptotic pathway that often requires RNA and protein synthesis. The exact role of apoptosis in Alzheimer's disease is unknown but it is suggested that the deposition of B amyloid protein in the brain might prevent the neurons from receiving the trophic factors or make them more susceptible to the cytotoxic effects of excitatory amino acids.

Apoptosis provides an additional means of regulating cell number and so also biological activity and unlike simple degeneration, death that is dependent on active participation of cellular components can be partially suppressed. Elucidation of the mechanism of apoptosis should provide new targets of therapy and help to design new drugs to certain degenerative diseases or to preventing death of acutely stressed cells or preventing death in cells undergoing the kind of long term stress as in Alzheimer's disease.

Apoptosis in therapy :

Pharmacological manipulation of apoptosis will make it possible to regulate the aging process and other diseases by increasing or decreasing the susceptibility of particular cells to apoptosis. Apoptosis should prove a new approach to chemotherapy of epithelial cancers and to design new drugs to some degenerative diseases. Exploration of the alternative idea that tumor cells could be eliminated by artificially triggering death through apoptosis seems attractive.

Drugs that promote apoptosis could amplify the effects of cancer chemotherapeutic agents on resistant cells for e.g. by inhibiting the expression of bcl-2. Inhibitors of apoptosis could be useful for AIDS, to treat diseases caused by too much cell death e.g. Parkinson's disease or diseases with long term stress such as Alzheimer's disease. Gene therapy could be beneficial to degenerative diseases. Apoptosis advocates hope to conquer cancer besides, ischemia, stroke, heart disease and autoimmunity in the near future.

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